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Characterization of genotypic response of different chickpea (*Cicer arietinum* L.) genotypes against chickpea blight (*Ascochyta rabiei*)

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Abstract

Chickpea blight is one of the most destructive diseases of chickpea (*Cicer arietinum* L.) worldwide caused by *Ascochyta rabiei* (Pass.) Lab. This disease shows comparatively more devastating effects in cold and humid ecological conditions. During epidemic conditions in deserts it results in great damage by spreading at the entire crop level. To alleviate this setback, several interventions are practiced. However, breeding for resistance to the pathogen is the most suitable and environmentally conducive strategy. The present study was conducted to find out good performing germplasm against chick blight and to study the pathogenic effect of the pathogen. The experiment was conducted by using randomized complete block design by following three replications and three treatments (T_o with no spores, T_1 with approximately 8350 spores/ml and T_2 with approximately 16700 spores/ml) sprayed to induce disease condition. Among total six genotypes, three kabuli and three desi genotypes were screened. Disease symptoms were recorded at international rating scale 1-9. In kabuli genotypes, significant response was observed in treatments T_1 and T_2. With the increase in number of spores/ml i.e. increased from T_1 to T_2, kabuli genotype named K-01248 move on rating scale from resistant to tolerant and genotype named K-01250 from tolerant to moderately susceptible. Interestingly Desi genotypes had showed high resistance in all treatments as compared to the kabuli genotypes. These highly resistant desi genotypes can be utilized in further breeding program for development of resistant high yield genotypes.

Keywords: Chickpea, Chickpea Blight, Genotypes, Screening, Breeding.

Introduction

Chickpea (*Cicer arietinum* L.) is the third most important pulse crop around the globe (Sarwar *et al*., 2012; Hirich *et al.*, 2014; Shah *et al.*, 2015; Khan *et al.*, 2018) after soybean and peas (Malik *et al.*, 2011, Jabbar *et al*., 2014). Chickpea attains importance as it stipulates as a source of nutrition for humans and animals. Furthermore, chickpeas have high protein (8.86%) and carbohydrates (27.42%) contents which constitutes about major portion of the total dry seed weight (USDA). Its cultivation spread from the Mediterranean basin to the Indian sub-continent and south-ward of Ethiopia and East African highlands. In Pakistan most of the rain-fed areas remain under chickpea cultivation during rabi season. In Pakistan, chickpea quantitatively takes as small portion and qualitatively as food supplement for the vegetarian diet (Jabbar *et al*., 2014). During the year 2018-19, an increase up to 35.6% was observed in the chickpea yield than the previous year due to prevalence of optimum climatic conditions at the time of sowing (Economic Survey 2018-2019). Besides of quality protein, chickpea has nitrogen fixing ability (Sahi *et al.*, 2012) which ultimately increases the soil fertility when use in different cropping systems (Shah *et al.*, 2015). According to a survey, chickpea alone contributes about 76% of the total pulses grown in Pakistan (Agriculture economic survey 2017-2018). Similar to other crops, chickpea is also prone to many biotic and abiotic stress factors which affect its production (Pande *et al*., 2005). Among those, chickpea blight is one of the most devastating one. The causal organism of Chickpea blight is *Ascochyta rabiei* (Pass.) Lab. In severe cases chickpea blight may cause yield loss up to 100 % (Pande *et al*., 2005). Chickpea blight epidemics between 1980–1984 caused heavy yield losses in Pakistan (Bashir and Ilyas, 1986). Regardless of losses in the past, annually this disease is causing 20–25% yield losses in Pakistan (Jamil *et al*., 2010).

Currently chickpea blight is controlled by different fungicides. For huge areas application of fungicides is expensive and toxic for the environment which makes it one of the unsuitable options for the disease control. Furthermore, resistance against those fungicides can be induced in *A. rabiei* due to imprudent use of these fungicides which may results in the development of new pathotypes or isotypes of the fungus. Development of resistant cultivars through breeding (Ilyas *et al*., 2007; Sarwar *et al*., 2012) and modern biotechnological tools (transgenics) can be one of the best options to overcome from

this problem. As we know that in most of the countries transgenic crops are banned. So, the most appropriate option to overcome from this problem is the development of resistant cultivars through conventional crop breeding. Because of the development of new isotypes and pathotypes most of the already approved high yielding cultivars became susceptible to chickpea blight (Jamil *et al*., 2010). Hence, identification of durable resistant advanced lines is inevitable to find some good sources of resistance against this disease. Therefore, present study was designed to assess the pathogenic effect on different genotypes and to screen the resistant genotypes against chickpea blight for future breeding programs.

Materials and Methods

Experiment location and Germplasm

Experiment was conducted in the University of Agriculture, Faisalabad (UAF), Pakistan during the year 2017-18. Six genotypes (3 Kabuli and 3 Desi) were obtained from Pulses Research Institute (PRI), Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. Details of genotypes are shown in the table 1.

Furthermore, highly susceptible chickpea variety Punjab-1 (PB-1) was planted as a spreader to produce the disease pressure after every two lines. Randomized complete block design (RCBD) with three replications and three treatments was followed.

Fungal Material

For the fungal development method of Shah *et al.,* 2015 was used. Isotypes of *Ascochyta rabiei* used in the experiment was isolated from infected stems, sheath and chickpea pods. Sterilization was done in 5% sodium hypochlorite for 2 minutes. The samples were then dried on sterilized filter paper which further placed on Potato Dextrose Agar (PDA) for fungal colonization. Incubation was done at 20^{o}C±2 with 12 hours light/dark cycle for seven days to ensure optimum fungal growth. Developed colonies were then sub-cultured on chickpea seed meal agar (CSMA) media. Streptomycin was used in petri dishes to grow free fungus *A. rabiei* before pouring CSMA media (Sahi *et al*., 2012). Then samples were incubated for two weeks for proper colony development. At the end fungal colonies were developed with pycnidia.

Preparation of inoculum and experimental conditions

Two inoculums were prepared by adding fungal spores in sterilized water. Spores were counted by Hemocytometer. Treatment (T_o) used as check with no inoculum spray, treatment (T_1) was inoculated with approximately 8350 spores/ml and treatment (T_2) was inoculated with approx. 16700 spores/ml. Fresh prepared inoculums were used for spray until blight symptoms appeared. Inoculation was continued until susceptible spreader (PB-1) showed the symptoms of severe conditions i.e. death of spreader line. The inoculums were sprayed at mid pod stage of chickpea. Throughout the experiment, all recommended agronomical practices were followed to raise the crop. During experiment 80-90% humidity was maintained by tap water spraying in the morning and evening. To check humidity hygrometer was used. Polythene sheet was used for the isolation of Treatment (T_1) and (T_2).

Rating Scale

Disease was estimated when pods of susceptible variety(s) was completely infected. Infection was classified as shown in the table No. 2 (Manjunatha and Saifulla, 2013).

Results

Results based on susceptibility and resistance of genotypes is summarized in table-3. Susceptible PB-1 showed severe symptoms of disease at mid pod stage. Results showed more resistance in desi as compared to Kabuli genotypes. In control condition (T_o), all lines were healthy and showed no blight symptoms. In treatment (T_1) with approx. 8350 spores/ml, Kabuli chickpea genotypes K-01248 showed resistance, K-01250 proved as tolerant and K-01216 was estimated as moderately susceptible. Interestingly desi chickpeas genotypes showed no sign of lesions as shown in table-3. In treatment (T_2) with approx. 16700 spores/ml, Kabuli chickpea genotypes K-01248 observed as tolerant, K-01250 as moderately susceptible and K-01216 as moderately susceptible to susceptible whereas desi chickpea genotypes showed no lesions (Table-3).

Discussion

Most of the researchers identified resistant genotypes by evaluation of germplasm against blight in field conditions (Bashir *et al.,* 2006; Iqbal *et al.,* 2010 and Sarwar *et al.,* 2012). The most important aspect of integrated disease management strategy is the use of resistant cultivars. Untill now very low frequency of resistance to *A. rabiei* has been found in the present chickpea germplasm (Sarwar *et al.,* 2012). Another approach can be the use of fungicides to control the disease which is expensive and not feasible environmentally as well. So the identification of resistant chickpea genotypes against *A. rabiei* can be one of the best options to overcome from this problem (Akhtar *et al.,* 2009). Moreover when new races of pathogen appear than lose in resistance of resistant cultivars is observed (Jabbar *et al.,* 2014).

In present study, resistance in the desi chickpea genotypes was observed based on the rating scale, but in case of Kabuli genotypes (K-01248) showed tolerance in T_2. Present study has also explained the significant response of the

Kabuli genotypes against the conditions which are favorable for disease spread at pod stage. In a variety "Punjab-1" maximum number of lesions were observed when inoculated with the *A. rabiei* spores (Sahi *et al.,* 2012). Desi chickpea genotypes proved more resistance as compared to Kabuli ones (Shah *et al.,* 2015). Our study supported these results with the findings that Punjab-1 showed the severe symptoms of blight upon inoculation and desi chickpea genotypes had more resistance as compared to Kabuli chickpea genotypes. Desi chickpea germplasm i.e. Punjab-2008, bittal-2016 and D-09027 have no infection (0%) of disease. These results depicted that desi chickpea genotypes had more resistance against blight disease as compared to Kabuli genotypes.

Conclusion

Chickpea blight is one of the most destructive diseases of chickpea which causes severe yield loses. Use of fungicides is not a useful method to control disease. Present study explained significant variation among genotypes according to the above-mentioned scale of resistance that can be used as direct source of hybridization for the transfer of resistant genes into high yielding susceptible genotypes. Results showed that desi genotypes are more promising against the disease. In the future, these desi genotypes can be used in the breeding programs for the development of new resistant genotypes against chickpea blight.

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Table 1. Genotypes assessed in the study

Sr. No.	Kabuli Genotypes	Desi Genotypes
1	K-01250	Punjab-2008
2	K-01216	Bittal-2016
3	K-01248	D-09027

Table 2. Classification of infection according to the rating scale

Sr. No	Scale	Infected Plants (%)	Disease Infection
1	1	0	No infection
2	2	1-5	Highly resistant
3	3	6-10	Resistant
4	4	11-15	Moderately resistant
5	5	16-40	Tolerant
6	6	41-50	Moderately susceptible
7	7	51-75	Moderately susceptible to susceptible
8	8	76-100	Highly susceptible

(Source: Nene, 1984)

Table 3. Classification of infection in the tested genotypes.

T_o (No inoculation)		T_1 (approx.. 8350 spores/ml)		T_2 (approx.. 16700 spores/ml)	
Scale	**Genotypes**	**Scale**	**Genotypes**	**Scale**	**Genotypes**
No infection (0%)	Kabuli (K-01250) (K-01216 and K-01248)	Resistant (6-10%) Tolerant (16-40%)	Kabuli K-01248 K-01250	Tolerant (16-40%) Moderately Susceptible (41-50%)	Kabuli (K-01248 K-01250
No infection (0%)	Desi (Punjab-2008, Bittal-2016 and D-09027)	Moderately Susceptible (41-50%) No infection (0%)	K-01216 Desi (Punjab-2008, Bittal-2016, D-09027)	Moderately Susceptible to Susceptible (51-75%) No infection (0%)	K-01216) Desi (Punjab-2008, Bittal-2016, D-09027)